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13. ABSTRACT *(Maximum 200 words)*

Bacteriorhodopsin, from the purple membrane (PM) of *Halobacterium halobium*, was chemically modified with methoxypolyethylene glycol (MW = 5000) succinimidyl carbonate. The polyethylene glycol-bacteriorhodopsin (m-PEG-SC-BR33) conjugate, containing one PEG chain, was water soluble. The secondary structure of the conjugate in water appeared partially denatured but was shown to contain α-helical segments

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by circular dichroism (CD) spectroscopy. The isolated bacteriorhodopsin conjugate, with added retinal, was refolded in a mixed detergent-lipid micelle and had an absorption maximum at 555 nm. The refolded conjugate was transferred into vesicles which pumped protons, upon illumination, as efficiently as did native BR. Modification of the PM with methoxy-polyethylene glycol did not alter the native structure or inhibit proton pumping, and therefore it is suggested that the glycol polymer is present as a covalently linked moiety to residues unnecessary for proton pumping and proper folding. The site of attachment of mPEG was determined to be either at Lys 129 or Lys 159, with position Lys 129 the most probable site of attachment. The m-PEG-SC-BR33 could be stepwise refolded to the native conformation by the addition of trifluoroethanol to lower the dielectric constant, simulating the insertion of the BR into the phospholipid bilayer.

CONFORMATION OF MEMBRANE PROTEINS: BACTERIORHODOPSIN

FINAL REPORT

GERALD D. FASMAN

MAY 13, 1994

U.S. ARMY RESEARCH OFFICE

CHEMICAL AND BIOLOGICAL SCIENCES DIVISION

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4. REPORT

A. STATEMENT OF PROBLEM

Bacteriorhodopsin (BR) is an integral membrane protein (248 amino acids) which catalyzes the light-induced proton translocation across the membrane of *Halobacterium halobium*. Research was directed toward: (1) the elucidation of the folding path of BR as it attains its final conformation in the membrane, and, (2) to facilitate goal #1, a water-soluble derivative, a methoxy polyethylene glycol derivative (m-PEG-BR) will be synthesized.

B. SUMMARY OF RESULTS

Bacteriorhodopsin, from the purple membrane (PM) of *Halobacterium halobium*, was chemically modified with methoxypolyethylene glycol (MW = 5000) succinimidyl carbonate. The polyethylene glycol-bacteriorhodopsin (m-PEG-SC-BR33) conjugate, containing one PEG chain, was water soluble. The secondary structure of the conjugate in water appeared partially denatured but was shown to contain α-helical segments by circular dichroism (CD) spectroscopy. The isolated bacteriorhodopsin conjugate, with added retinal, was refolded in a mixed detergent-lipid micelle and had an absorption maximum at 555 nm. The refolded conjugate was transferred into vesicles which pumped protons, upon illumination, as efficiently as did native BR. Modification of the PM with methoxy-

polyethylene glycol did not alter the native structure or inhibit proton pumping, and therefore it is suggested that the glycol polymer is present as a covalently linked moiety to residues unnecessary for proton pumping and proper folding. The site of attachment of mPEG was determined to be either at Lys 129 or Lys 159, with position Lys 129 the most probable site of attachment. The m-PEG-SC-BR33 could be stepwise refolded to the native conformation by the addition of trifluoroethanol to lower the dielectric constant, simulating the insertion of the BR into the phospholipid bilayer.

C. PUBLICATIONS

1. "The Refolding and Proton Pumping Activity of a Polyethylene Glycol-Bacteriorhodopsin Water-Soluble Conjugate," G. Fasman and G. Sirokmán. Sixth Symp. of the Protein Society, 1992.

2. "Refolding and Proton Pumping Activity of a Polyethylene Glycol-Bacteriorhodopsin Water-Soluble Conjugate," G. Sirokmán and G.D. Fasman. *Protein Science* **2**, 1161-1170 (1993).

3. "Crystallizing Membrane Proteins. A New Successful Method," G.D. Fasman and G. Sirokmán, *FASEB J.* **8**, Abst. 1113 (1994), Amer. Soc. Biochem. and Mol. Biol., 1994 Meeting, Washington, D.C.

D. PARTICIPATING SCIENTIFIC PERSONNEL

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5. NO INVENTIONS